PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Charli KRUSE

Serial No: 10/561,628 Group Art Unit: 1651

Filed: 02-09-2006 Examiner: BARNHART, LORA E.

Att. Docket No.: B1180/20049 Confirmation No.: 8428

For: METHOD FOR DIFFERENTIATING STEM CELLS IN CELLS THAT PRODUCE A PANCREATIC HORMONE

DECLARATION OF CHARLI KRUSE, PH.D. UNDER 37 CFR § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Charli Kruse, Ph.D., a citizen of Germany, hereby declare and state:

1. The resume attached as Exhibit A accurately reflects my professional credentials.

2. I am the sole inventor named in the above-identified application.

3. My research is funded in part by Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V., the assignee of the above-identified application.

4. I understand from my review of the Office Action of 10/21/2008 that claims 1-17 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for a method including obtaining *bona fide* pluripotent stem cells from exocrine tissue, and methods of making pancreatic hormone-producing cells from exocrine tissue pluripotent stem cells.

5. I understand from attorneys for the assignee that the standard for determining compliance with the enablement requirement of Section 112, first paragraph, is whether the

original specification contained sufficient information regarding the subject matter of the claims as to enable one reasonably skilled in the pertinent art to make and use the claimed invention without undue experimentation. While I am not an expert in patent law, my experience and educational background, particularly as a researcher and private lecturer at the University of Lübeck, enable me to render an informed opinion as to the facts underlying the determination of enablement, including the level of ordinary skill in the art, information known in the art at the time of the invention, and what constitutes undue experimentation to one of ordinary skill in the art. For the reasons discussed below, I believe that the application is in compliance with the enablement requirement of Section 112, first paragraph, and I show that the full scope of claims 1-17 is enabled by the specification such that no undue experimentation to prepare and/or practice the invention is required.

6. The application describes how isolated pluripotent adult stem (IPAS) cells are obtained from exocrine glandular tissue. Using the isolation methods described in the application, I and/or researchers under my direct supervision produced a variety of different IPAS cells from species additional to rats and humans, and from exocrine glandular tissue additional to pancreatic tissue, from which cells from mice, rats, humans and from pancreas, salivary glands or skin formations were well characterized.

7. The data in Table 1 below show that the invention is enabled for more than just IPAS cells from rat and human pancreatic tissue.

Table 1. Different isolated stem cell lines from exocrine glands.

Species	Organ	Number of Cell lines
Goat	Pancreas	1
	G. Submandibularis	1
	G. Parotis	1
Mouse	Pancreas	14

	G. Submandibularis	1
Human	Pancreas	6
	G. Submandibularis	2
	G. Parotis	3
	Glandulae Buccales	1
Rat	Pancreas	12
Roe Deer	Pancreas	4
Watussi Cattle	Pancreas	1
Platalea Leucorodica (A Heron)	Pancreas	1
Domestic Pig	Pancreas	1
Wild Pig	Pancreas	3
African Green Monkey	Pancreas	1
Guanaco	Pancreas	1
Vietnamese Sica Deer (Cervus Nippon Pseudotaxis)	Pancreas	3
White Naped Crane (Grus Vipio)	Pancreas	1
Chicken	Pancreas	2

8. The invention has enabled IPAS cells to be isolated from human sweat glands (glands without acini) as evidenced by the stem cell marker nestin, which marker has been described by Wiese et al., Cellular and Molecular Life Sciences 2004 Oct; 61(19-20):2510-22. See Figures below, which show nestin-positive staining (red) of human sweat glands (Figures A and B) and nestin-positive cells (Figure C), isolated from a human skin and sweat gland preparation.

9. In addition, I and/or technicians under my direct supervision obtained IPAS cells from African Boer Goats as described below.

10. Exocrine glandular tissue from the pancreatic tissue of African Boer Goats was prepared and treated as described in the specification of U.S. App. Serial No. 10/561,628 in order to isolate pluripotent adult stem cells therefrom. After cultivating the stem cells in cell culture for 3 passages, the resulting stem cells were seeded and the differentiated cells derived therefrom

were stained with antibodies against specific cell markers.

11. The differentiated cells stained positive for several cell markers having specificity for different cells of all 3 germ layers, as shown in the Figures attached as Appendix B. The differentiated cells stained positive for the ectodermal cell markers GFAP and neurofilaments (see Figure 1A and 1B). The differentiated cells stained positive for the mesodermal markers collagen-II and α-smooth muscle actin (see Figure 2A and 2B). The differentiated cells stained positive for the endodermal marker cytokeranin 18 and amylase (see Figure 3A and 3B).

12. With respect to the confirmation of a normal karyotype, submitted herewith are the results obtained by an independent cytogenetic laboratory in Kaiserslautern, Germany (attached as Appendix C). The findings of the independent cytogenetic laboratory are set forth in the summarizing opinion (see section labeled "Beurteilung", Appendix C) with respect to the specimen (translated form the German):

> Numerically and structural inconspicuous female karyotype, the satellite extension at one chromosome 22 is a normal variation without pathologic relevance.

13. Furthermore, I and/or technicians under my direct supervision obtained IPAS cells from African Boer Goats as described below.

14. Exocrine glandular tissue from the salivary glands of African Boer Goats was prepared and treated as described in the specification of U.S. App. Serial No. 10/820,430 in order to isolate pluripotent adult stem cells therefrom. After cultivating the stem cells in cell culture for 19 passages, the resulting cells were seeded and the stem cells and differentiated cells derived therefrom were stained with antibodies against specific cell markers, as shown in the Figures attached as Appendix D. The stem cells stained positive for two stem cell markers (see Figure

4A and 4B) and the differentiated cells stained positive for several cell markers having specificity for different cells of all 3 germ layers: ectoderm (see Figure 5A, 5B and 5C), mesoderm (see Figure 6A and 6B) and endoderm (see Figure 7A and 7B).

15. Accordingly, a person reasonably skilled in the art would have been enabled by the original disclosure to isolate IPAS cells from a variety of cell types from a variety of organisms without undue experimentation.

16. I understand from my review of the Office Action of 10/21/2008 that claims 1-17 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement.

17. I understand from attorneys for the assignee that the standard for determining compliance with the written description requirement of Section 112, first paragraph, is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, Applicants were in possession of the invention as now claimed. For the reasons discussed in paragraphs 1-15, I believe that persons skilled in the art would have understood from the original specification that Applicants are entitled to claim a method IPAS cells from a variety of cell types from a variety of organisms.

18. Accordingly, a person reasonably skilled in the art would understand that Applicants were in possession of each of the claimed embodiments.

* * *

I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States

Application No. 10/561,628

Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 25.05.2009

Charli Kruse, Ph.D.

Appendix A

Curriculum Vitae

PD Dr. Charli Kruse

Group Manager of the group "Cell Differentiation & Cell Technology" of the Fraunhofer Institute of Biomedical Engineering, MFC/ICL, Maria-Goeppert-Straße 1, D-23562 Lübeck, Germany

born:	July, 17. 1960 in Wismar/Germany
1982-1987	Study Marine Ecology, University of Rostock
1986-1987	Diploma Thesis at the Institute of Animal Physiology at the University of Rostock
1987-1991	Scientific Assistant at the Institute of Animal Physiology at the University of Rostock
1992	Dr. rer. nat (PhD), University of Rostock
1991-2001	Scientific Assistant (PostDoc) at the Institute of Medical Molecular Biology at the University of Luebeck
2000	Habilitation, University of Luebeck
2000	Venia legendi, University of Luebeck
2001-2005	Associate Professor (C2), University of Luebeck
since 2004	Head of the FhG-IBMT-Group "Cell Differentiation & Cell Technology" at the University of Luebeck
since 2005	Academic Senior Councillor (A14), University of Luebeck

Research Topics: haematology of fishes
physiology of fishes
intracellular transport mechanisms
RNA-protein interaction
cellular biology
stem cell biology

Selected publications in the field of cell manipulation and stem cell biology

1. Kruse, Ch.; Strehlow, B.; Schmidt, H. and Müller, P. K. (1996) Presence of trypsin in distinctive body segments of leptocephalus larvae of Anguilliformes. Aquaculture; 142, 237-244
2. Kügler, S.; Grünweller, A.; Probst, C.; Müller, P. K. and Kruse, C. (1996) Vigilin contains a functional nuclear localisation sequence and is present both in the cytoplasm and the nucleus. FEBS Letters; 382, 330-334
3. Kruse, C., Willkomm, D., Grünweller, A., Vollbrandt, T., Sommer, S., Busch, S., Pfeiffer, T., Brinkmann, J., Hartmann, R.K., and Müller, P. K. (2000) Export and transport of tRNA are coupled to a multi-protein complex. Biochemical Journal; 346, 107-115
4. Kruse, C., Hartmann, R. K. and Müller, P. K. (2001) Nuclear-cytoplasmic translocation of tRNA; Exp. Cell Res., 262, 3-7
5. Kruse, C., Willkomm, D., Gebken, J., Schuh, A., Stoßberg, H., Vollbrandt, T. and Müller, P.K. (2003) The multi-KH-protein vigilin associates with free and membrane

bound ribosomes. Cell. Mol. Life Sci.; 60, 2219-2228
6. Kruse, C., Birth, M., Rohwedel, J., Assmuth, K., Goepel, A., and Wedel, T. (2004) Pluripotency of adult stem cells derived from human and rat pancreas. Appl. Phys. A 2004; 79, 1617-1624
7. Kruse, C., Bodo, E., Petschnik, A. E., Danner, S., Tiede, S. and Paus, R. (2006). „Towards the development of a pragmatic technique for isolating and differentiating nestin-positive cells from human scalp skin into neuronal and glial cell populations: generating neurons from human skin?" Exp. Dermatol.; 15, 794-801
8. Kruse, C., Kajahn, J., Petschnik, A. E., Maaß, A., Klink, E., Rapoport D. H. and Wedel, T. (2006). Adult pancreatic stem/progenitor cells spontaneously differentiate in vitro into multiple cell lineages and form teratoma-like structures. Ann. Anat.; 188, 503-517
9. Guldner, N. W., Kajahn, J., Klinger, M., Sievers, H.-H. and Kruse, C. (2006) Autonomously contracting human cardiomyocytes generated from adult pancreatic stem cells and enhanced in cocultures with myocardial biopsies. Int. J. Artif. Org.; 29: 1158 - 1166
10. Danner, S., Kajahn, J., Geismann, C., Klink, E. and Kruse, C. (2007) Derivation of oocyte-like cells from a clonal pancreatic stem cell line. Mol. Hum. Reprod.; 13: 11-20. Epub 2006 Nov 17
11. Tiede, S., Kloepper, J.E., Bodò, E, Tiwari, S., Kruse, C. and Paus, R. (2007) Hair follicle stem cells: Walking the maze, Eur. J. Cell Biol. (in press)

Appendix B

Figure 1. Ectodermal Marker (GFAP, Neurofilaments, Cytokeratin)

Figure 1A. Stained with antibodies against GFAP and with DAPI

Figure 1B. Stained with antibodies against neurofilaments and with DAPI

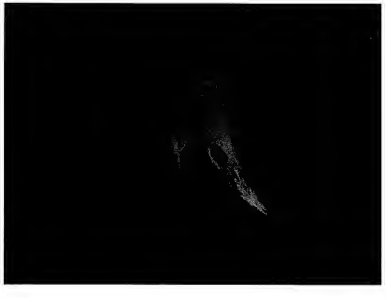

Figure 2. Mesodermal Marker (Collagen-II, SMA)

Figure 2A. Stained with antibodies against Collagen-II and with DAPI

Figure 2B. Stained with antibodies against α-smooth-muscle-actin and with DAPI

Figure 3. Endodermal Marker (Cytokeratin 18, Amylase)

Figure 3A. Stained with antibodies against Cytokeratin 18 and with DAPI

Figure 3B. Stained with antibodies against Amylase (green) and with DAPI

Appendix C

INSTITUT FÜR IMMUNOLOGIE UND GENETIK
am Klinikum Kaiserslautern

LABORARZTPRAXIS
Dr. med. Bernhard Thiele
Facharzt für Immunologie
Facharzt für Laboratoriumsmedizin

Wissenschaftliche Beratung:
Prof. Dr. med. Wolfram Henn
Facharzt für Humangenetik

Institut für Immunologie und Genetik, Transplantationsimmunologie Kaiserslautern
67613 Kaiserslautern Postf. 2565, Hellmut-Hartert-Strasse 1 67655 Kaiserslautern

Tel. 0631 316700 Fax: 0631 3167020
E-mail: immungenetik@t-online.de

Inst.f.Med.Molekularbiologie d. Universität

PD Dr. Charli Kruse

Ratzeburger Allee 160
23552 Lübeck

Name:	Lübeck Zellkultur
Geb.:	SSW:
Labornummer:	04-18112
Eingangsdatum:	23.04.04
Befunddatum:	04.05.04
Pat.Nummer:	
Seite:	1/1

ZYTOGENETISCHER BEFUNDBERICHT

Material: Zellkultur

Chromosomenanalyse

Metaphasen - numerisch/grobstrukturell:	21 (1x44, 2x45, 18x46 Chromosomen)
Metaphasen - feinstrukturell:	10
Bänderungstechnik:	GTG-Banden
Bandenauflösung (ca.):	450
Karyotyp(en):	46,XX,22s+ [cp10]

Beurteilung:

Numerisch und strukturell unauffälliger weiblicher Karyotyp, bei der Satellitenverlängerung an einem Chromosom 22 handelt es sich um eine Normvariante ohne pathologische Relevanz.

Dr. med. B. Thiele

Endbefund

Appendix D

Figure 4. Stem cell marker (Oct-4 und Nanog)

Figure 4A. Stained with antibodies against Oct-4

Figure 4B. Stained with antibodies against Nanog

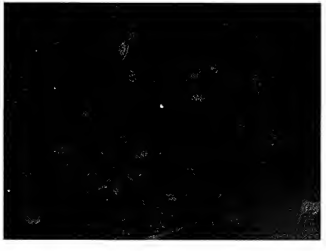

Figure 5. Ectodermal Marker (GFAP, neurofilaments, Cytokeratin)

Figure 5A. Stained with antibodies against GFAP and with DAPI

Figure 5B. Stained with antibodies against Cytokeratin and with DAPI

Figure 5C. Stained with antibodies against neurofilaments and with DAPI

Figure 6. Mesodermal Marker (Collagen-II, SMA)

Figure 6A. Stained with antibodies against Collagen-II and with DAPI

Figure 6B. Stained with antibodies against α-smooth-muscle-actin and with DAPI

Figure 7. Endodermal Marker (Insulin, Amylase)

Figure 7A. Stained with antibodies against Amylase und with DAPI

Figure 7B. Stained with antibodies against Insulin and with DAPI